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# Effect of Gamma Irradiation on Intestinal Crypts Survival in Mice Pretreated with N-Nitrosodiethylamine

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## Abstract

A new mathematical equation has been developed by which whole-body exposure to gamma radiation may be used to predict the survival characteristic of the intestinal crypt in mice pretreated with *N*-nitrosodiethylamine (NDEA). A computer fitting procedure showed the best agreement between predicted and observed curves. The results suggest that fewer cells may survive in crypts after gamma irradiation of NDEA-treated mice.

**Keywords:** gamma radiation, *N*-nitrosodiethylamine, intestinal crypts

## Introduction

A variety of chemical carcinogens and ionizing radiation have been used in the past to study proliferation processes in the intestinal crypt [1, 2, 3]. In our previous experiments, the intestinal crypts have been shown to be very sensitive targets of nitrite-induced toxicity [4]. Grudzihski has recently presented a dose-response equation to predict the number of surviving cells in crypts of gamma irradiated mice poisoned with sodium nitrite and potassium nitrate [5, 6].

Since only scant data on this problem has been specifically addressed to the question of *N* -nitrosoamine-induced toxicity in crypts [7, 8], it was decided to undertake the next investigation to establish crypt survival in mice pretreated with *N* -nitrosodiethylamine (NDEA). Gamma irradiation was used to determinate the functional ability of crypts for survival. The present work is also a study of a mathematical equation, by which whole-body exposure to gamma radiation associated with NDEA may be used to measure the survival characteristic of intestinal crypts.

## Material and Methods

### Animals and Treatment

Male B6C3F1 (C57BL/6 x C3H/w) mice aged 10-12 weeks were used in the studies. Before the experiment, the animals were acclimatized for 14 days under standard conditions (ambient temperature 22 ± 2°C, air humidity 40-70%, light-darkness cycle 12/12 h). Throughout the experiment, the mice were given a standard diet (Murigran pellet, Motycz, Poland) and water *ad libitum.* The animals were divided into 4 groups of 30 mice each. An aqueous solution of W-nitrosodiethylamine (NDEA) (E. Merck AG, Darmstadt, Germany) was administered *per os* to mice at a daily dose of 0.01, 0.1, 1.0 or 5.0 mg NDEA/kg body weight for 21 days. Control mice received saline only. The animals were irradiated at 12 hours (day 22) after the last NDEA and/or saline pretreatment using a cobalt-60 γ-irradiator, which delivered a dose rate of about 2.0 Gy per minute. Briefly, the unanesthetized mice were immobilized in plastic boxes and immediately exposed to 2.5, 5.0, 7.5 or 10.0 Gy of

whole-body gamma irradiation (6 animals per dose of irradiation). The total radiation dose was monitored using the DM-82 clinical dose rate mater. The unevenness of the distribution of radiation dose in the whole-body-irradiated animals was below 5%. The mice were sacrificed by cervical dislocation at 78 hours after gamma irradiation, and the small intestine was removed from the mice and rinsed with cold saline. The small pieces of intestine (5-6 cm) were fixed in Carnoy's fixative for 25 minutes, and cut into 5 μm thickness after paraffin embedding and stained with Harris hematoxylin and eosin. The total number of cells per crypt section was estimated quantitatively under light microscope using the method of Withers and Elkind [9].

### Evaluation Methods and Statistics

The crypt surviving fraction was approximated by the following equation:

$$S(D) = 1 - \{1 - [\exp(\delta - \alpha_1 D_r + \alpha_2 D_r^2 - \beta_1 D_{ch} + \beta_2 D_{ch}^2)]\}$$

where $D_r$ is the total dose of γ-radiation (Gy), $D_{ch}$ is the total dose of NDEA (mg/kg body weight), $\alpha_1$, $\alpha_2$ are the γ-radiation curve coefficients ($Gy^{-1}$, $Gy^{-2}$), $\beta_1$, $\beta_2$, are the NDEA curve coefficients (mg/kg body weight)$^{-1}$, (mg/kg body weight)$^{-2}$, 5 is the associated radiation/NDEA hybrid coefficient, the constant value for each S(D).

In the present experiment, it was assumed that crypt survival was according to Poisson statistics, and that the dose-response curves obtained were a result of fitting the equation S(D) to the experimentally obtained data. For data analysis, the standard errors of crypt surviving fraction were estimated by propagating the standard errors in the crypt numbers and diameters of each of the treatments groups [10]. The standard errors estimated were based on the 50 sections assayed in each dose group. A linear regression for fit to the crypt survival as a function of radiation and NDEA doses per fraction was performed as a test for trend. A significance level of the 0.05 was used throughout. The Mann-Whitney U test was made to compare the estimated derived from all fitted curves. The Kriging's method was used to calculate the auto-correlation between experimentally data points produced and a minimum variance unbiased estimate.

## Results and Discussion

Figure 1A-1E show the experimentally obtained crypt surviving fraction S(D) as a function of γ-radiation and NDEA doses, respectively (open points). The results presented here are in agreement with those reported by others, and thus confirm the apparent decrease in crypt number with the radiation at doses used in this assay [11, 12]. The crypt survival parameters after irradiation in NDEA-treated mice were analyzed using a computer program [13, 14]. It was based on the idea described in details by Hendry and Potten [15] that

(i) one surviving cell is sufficient for complete crypt regeneration (which is inherent in the definition of clonogenic cells and

(ii) the clonogenic cells survive independently of one another.

Figures 1A-1E show the S(D) values which have been estimated for the corrected data fitted by the exponential equation (closed points). Fitting a radiation pre-equation to the experimental data of Figures 1A-1E provided estimates of $\alpha_0 = 4.9915 \times 10^{-3} \pm 1.4114 \times 10^{-4}$, $\alpha_1 = -5.3035 \times 10^{-2} \pm 2.5108 \times 10^{-3}$ $\alpha_2 = 6.6436 \times 10^{-4} \pm 0.7005 \times 10^{-5}$ (radiation fit without NDEA). Fitting a NDEA pre-equation to the experimental data of Figures 1A-1E provided estimates of $\beta_o = -1.1096 \times 10^{1} \pm 1.1348 \times 10^{-3}$, $\beta_1 = -2.4727 \times 10^{-1} \pm 2.2510 \times 10^{-3}$, $\beta_2 = 4.0328 \times 10^{-2} \pm 4.4629 \times 10^{-3}$, (NDEA fit without radiation). Fitting the final equation S(D) to the experimental data of Figures 1A-1E provided estimates of hybrid coefficient $\delta = \_ 1.7972 \times 10^{-1} \pm 1.5829 \times 10^{-3}$, and $\alpha_1 = 4.0245 \times 10^{-2} \pm 8.3628 \times 10^{-4}$, $\alpha_2 = 5.0414 \times 10^{-4} \pm 3.2618 \times 10^{-5}$ $\beta_1 = 2.0457 \times 10^{-1} \pm 1.5400 \times 10^{-3}$, $\beta_2 = 3.3353 \times 10^{-2} \pm 4.6183 \times 10^{-3}$ (radiation/NDEA fit). It should be emphasized that the transformed quantities of surviving cells

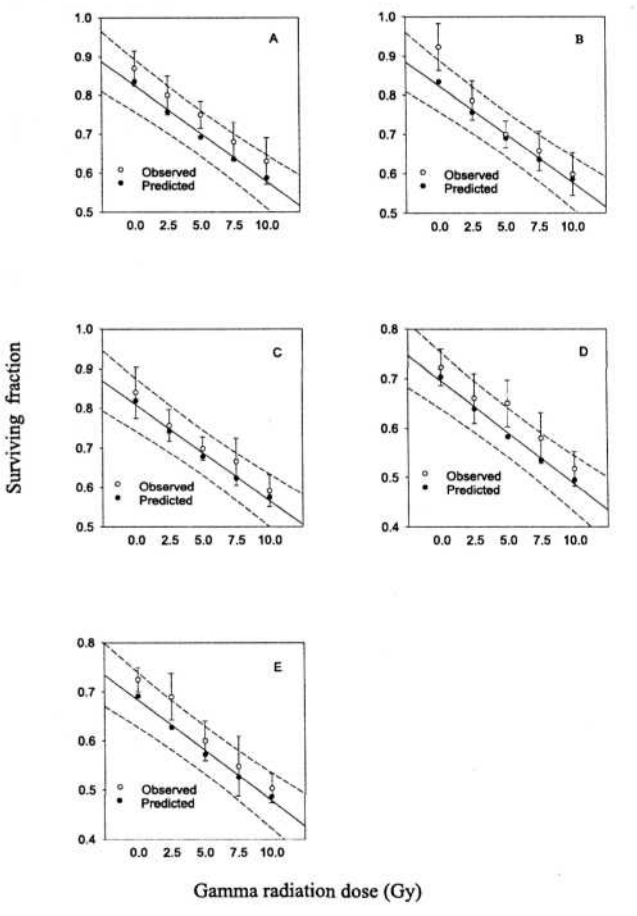


Fig. 1. The surviving fraction of intestinal crypts after gamma irradiation of saline- and NDEA-treated mice. The open points were plotted against the radiation doses after exposing mice to saline (A), 0.01 mg NDEA/kg (B), 0.1 mg NDEA/kg (C), 1.0 mg NDEA/kg (D), and 5.0 mg NDEA/kg (E), respectively. Mean ± SEM, n = 6 per point of irradiation (see evaluation methods and statistics). The solid line shows the linear regression of the predicted survival (solid points). The dashed lines are 95% confidence levels for the linear regression and describe the range within which the regression line values will fall a percentage of the time after repeated measurement.

per crypt structure capable of crypt regeneration suffer from a number of limitations in the NDEA-treated mice (without radiation). First, the Poisson correction of surviving crypts breaks down for survival S(D) = 0 and S(D) = 1, i.e. for these histological sections in which the observed proportion of surviving crypts is either 0% or 100%. A second limitation to the use of Poisson-transformed data is that when least-squares regression methods are employed to analyze the data, the statistical assumption of linear regression analysis is often not met. In other words, the scatter in replicate estimates of crypt cells is usually much larger in groups for which the proportion of surviving crypts is close to zero than it is for groups in which many crypts survive.

The present results show a decrease in the Do value, the mean lethal dose for crypt cells in small intestine [16]. As shown in Table 1, NDEA dosed to mice at 1.0 and 5.0 mg/kg body weight has been found to influence crypt radiation sensitivity. Such results are difficult to interpret, at present, because of the problem of defining a dose-response(s) at the target stem cells in contrast to administered NDEA dose [17]. Since the intestinal crypt cells in the steady state undergo a cycle of growth and budding, the large number of clonogenic cells cannot be employed unless there is widespread radio-toxicity in the crypt. It should be emphasized that both cell proliferation and crypt survival are stochastic processes; therefore, a small amount of increased cell replication in the crypt may occur which does not lead to detectable recovery of crypt cell population [18].

Table 1. Estimates of the mean lethal dose (Do) for crypt cells in small intestine after gamma irradiation of saline- and NDEA-treated mice.

| Treatment | The mean lethal dose, Do (Gy) | |
|---|---|---|
| | Observed | Predicted |
| Control (saline) | 1.923 ± 0.072 | 1.883 ± 0.115 |
| 0.01 mg NDEA/kg | 1.873 ± 0.152 | 1.805 ± 0.102 |
| 0.1 mg NDEA/kg | 1.833 ± 0.112 | 1.808 ± 0.123 |
| 1.0 mg NDEA/kg | 1.703 ± 0.092* | 1.623 ± 0.121* |
| 5.0 mg NDEA/kg | 1.693 ± 0.062* | 1.601 ± 0.132* |

* $p < 0.05$ as compared with control (saline).

In summary, there is considerable evidence that both experimentally and theoretically calculated data showed a decrease in the crypt survival of B6C3F1 mice. Our results indicate that N-mtrosodiethylamine increases the risk of radiation-induced injury in the small intestinal mucosa.

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